ANTICANCER RESEARCH *40*: 2209-2217 (2020)
doi:10.21873/anticanres.14182

Impact of Concomitant Medication Administered at the Time of Initiation of Nivolumab Therapy on Outcome in Non-small Cell Lung Cancer

MARTIN SVATON[1], MILADA ZEMANOVA[2], PETRA ZEMANOVA[2], JURAJ KULTAN[3], ONDREJ FISCHER[3], JANA SKRICKOVA[4], LENKA JAKUBIKOVA[4], MARKETA CERNOVSKA[5], MICHAL HRNCIARIK[6], MICHAL JIROUSEK[6], JANA KREJCI[7], DANIEL KREJCI[7], ONDREJ BILEK[8], JIRI BLAZEK[1], KAROLINA HURDALKOVA[9], MAGDA BARINOVA[9] and BOHUSLAV MELICHAR[10]

[1]*Department of Pneumology and Phthisiology, Charles University, Faculty of Medicine in Pilsen, Pilsen, Czech Republic;*
[2]*Department of Oncology, Charles University, First Faculty of Medicine in Prague, Prague, Czech Republic;*
[3]*Department of Respiratory Medicine, Palacky University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic;*
[4]*Department of Respiratory Diseases and TB, Faculty of Medicine, Masaryk University Brno and University Hospital Brno, Brno, Czech Republic;*
[5]*Department of Respiratory Medicine, First Faculty of Medicine of Charles University, Thomayer Hospital Prague, Prague, Czech Republic;*
[6]*Department of Pneumology, Charles University, Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czech Republic;*
[7]*Department of Pneumology, Charles University, Faculty of Medicine in Prague, Prague, Czech Republic;*
[8]*Department of Comprehensive Oncology, Masaryk Institute of Oncology, Faculty of Medicine, Masaryk University, Brno, Czech Republic;*
[9]*Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic;*
[10]*Department of Oncology, Palacky University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic*

Abstract. *Aim: To investigate potential association between administration of corticosteroids, antibiotics, probiotics, proton pump inhibitors, non-steroidal anti-inflammatory drugs (NSAID), statins and metformin and outcome in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. Patients and Methods: A total of 224 patients with advanced NSCLC treated at nine comprehensive cancer centers were analyzed in this national retrospective study. Survival statistics were evaluated using Kaplan–Meier method and Cox analysis. Results: Only corticosteroid use had a significant negative effect on the objective response rate. In the univariate analysis, there was no significant effect of the studied concomitant medications on the efficacy of nivolumab. In a subsequent multifactorial analysis, a possible positive effect of the concomitant use of NSAID at the initiation of nivolumab treatment was revealed. Conclusion: The results of the present retrospective exploratory analysis underscore the importance of knowing the exact type of concomitant medication, the route of administration, the dose of medication, and the region of the ongoing study. The present data indicated a significantly higher rate of progression in patients treated with corticosteroids and the possible positive effect of NSAID use at the initiation of nivolumab treatment.*

Correspondence to: Martin Svaton, Department of Pneumology and Phtisiology, Charles University, Faculty of Medicine in Pilsen, Husova 3, 301 00, Pilsen, Czech Republic. Tel: +420 377402973, e-mail: svatonm@fnplzen.cz

Key Words: Nivolumab, non-small cell lung cancer, corticoids, antibiotics, proton pump inhibitors, NSAID, statins, metformin.

Nivolumab is a human monoclonal antibody to programmed cell death protein 1 (PD1) that represents a new therapeutic option in the second-line treatment of advanced non-small-cell lung cancer (NSCLC). Improved efficacy with a more favorable adverse event profile has been documented for nivolumab compared to docetaxel in phase III trials. However, the objective response rate of nivolumab monotherapy is only about 20%, with the disease control rate

reaching approximately 50% (1, 2). Therefore, many patients do not benefit from nivolumab treatment and, considering the cost/efficacy ratio, identification of predictive parameters that would aid in patient selection for immunotherapy remains a topic of unmet medical need. Predictive biomarkers currently used include expression of programmed death-ligand 1 (PD-L1) and tumor mutation burden (3, 4). However, the predictive value of these biomarkers is far from being perfect, and many other, often unknown, parameters affect the efficacy of nivolumab, including biomarkers of inflammatory response and concomitant medications, as demonstrated in recent studies (5-8).

Corticosteroids are commonly used in patients with NSCLC to treat a range of conditions, *e.g.* symptomatic brain metastases and anorexia (9, 10). However, the immunosuppressive activity of corticosteroids obviously inhibits T-cell function (11). Corticosteroids can also modify the microbiome (12). Therefore, corticosteroid use may potentially impair the efficacy of immunotherapy. This hypothesis was first examined with regard to corticosteroid use during the treatment of immune-related adverse events, and was not confirmed (13), possibly due to the fact that the effect of corticosteroids on T-cell function is determined not only by the dose, but also by the timing of administration (14). Therefore, after T-cell activation induced by immunotherapy, effector cells may be protected from steroid-induced apoptosis (8). On the other hand, there are data indicating a deleterious effect of corticosteroid administered at the time of immunotherapy initiation on the efficacy of PD1/PD-L1 inhibitors (8, 15). However, a systematic review did not indicate a negative effect of corticosteroids on the outcome of treatment with PD1/PD-L1 inhibitors (16), and this hypothesis needs further investigation.

Antibiotics, probiotics and proton pump inhibitors (PPI) are also among the drugs that can affect the microbiome (17). Experimental work in mice highlighted the role of the gut microbiome in response to immunotherapy (18, 19). A subsequent study in humans also indicated a possible negative impact of antibiotics on the efficacy of immunotherapy (20). However, Kaderbhai *et al.* published discordant results (7). Similarly, the study reported by Hakozaki *et al.* did not demonstrate a significant effect of antibiotics on overall survival (OS) after nivolumab treatment in a multivariate analysis (17). Ahmed *et al.* hypothesized the possible existence of a difference between using broad-spectrum and narrow-spectrum antibiotics (21). Thus, the general effect of antibiotics on the efficacy of immunotherapy remains controversial.

PD1/PD-L1 blockade has also interactions with metabolic pathways (22-24). A possible effect of metformin (an antidiabetic agent) on potentiation of PD-L1 blockade was observed in a preclinical study (25). Metformin may also have a direct antitumor activity that has also been described for statins (26, 27). An off-target effect of the combination of metformin with statins resulting in longer survival in patients with NSCLC treated with chemotherapy was noted by Lee *et al.* (15). Similarly, a retrospective analysis reported by Omori *et al.* indicated a potential benefit of administering statins with nivolumab (8), with significantly improved overall response rate (ORR), but only a trend for improved OS. Therefore, these observations need further confirmation.

Non-steroidal anti-inflammatory drugs (NSAID) act, among other pathways, as cyclooxygenase 2 (COX2) inhibitors (28-30). COX2 is not detectable in most normal tissues, but is rapidly induced by inflammatory stimuli (31). COX2 is expressed across a range of solid tumor types and COX2 activity correlates with invasiveness and prognosis, reflecting an important role of COX2 in carcinogenesis and tumor progression (32). Prima *et al.* observed that COX2 and the prostaglandin E2 (PGE2) pathway regulate PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells (33). Moreover, an association between COX2 and PD-L1 expression was described in breast cancer and melanoma (34-36). A similar association was subsequently observed in colorectal cancer, where aspirin use was associated with significantly improved survival in patients with low PD-L1 expression (36). In addition, an association between COX2 expression and PD-L1 was also described in NSCLC, although the treatment of lung cancer cell lines with a COX2 inhibitor alone had no impact on PD-L1 expression in this study (37). Therefore, it is difficult to predict whether the concomitant use of NSAID (COX2 inhibitor) with nivolumab (PD-L1 inhibitor) might result in a synergistic effect.

Given this background, the aim of the present study was to investigate potential associations between corticosteroids, antibiotics, probiotics, PPIs, NSAIDs, statins and metformin and outcome in patients with NSCLC treated with nivolumab.

Patients and Methods

Study design and treatment. Clinical data of patients with cytologically or histologically confirmed advanced NSCLC treated with nivolumab were retrospectively analyzed. The patients were treated in the first- or higher line of treatment at nine oncology and pneumo-oncology Departments in the Czech Republic between 2015 and 2019. Nivolumab was administered intravenously at the approved doses of 3 mg/kg or a flat dose of 240 mg every 2 weeks. The treatment was administered until progression or unacceptable toxicity for a maximum of 2 years. In the case of treatment-related toxicity, administration of corticosteroids with/without interruption of nivolumab were recommended. Clinical follow-up including physical examination, chest X-ray and routine laboratory tests were performed at least every 4 weeks. Computed tomography (CT) or positron-emission tomography (PET)/CT were performed at regular intervals according to the local standards or when progression was suspected based on clinical or chest X-ray examination. Concomitant medications investigated in the present study included corticosteroids, antibiotics, probiotics, PPIs, NSAIDs, statins and

metformin. These concomitant medications were followed 1 month before and 1 month after the initiation of nivolumab treatment. Patients were considered treated with the above-mentioned agents if the treatment occurred any time during this 2-month period, and treatment beyond this period was considered only if started within this period to avoid potential bias in patients treated with nivolumab for prolonged duration. A national register TULUNG, a non-interventional post-registration database of epidemiological and clinical data of patients with advanced-stage NSCLC treated with targeted or biological therapies in the Czech Republic, served as the data source. The patients gave their informed consent to be included in this database and for use of these data for scientific purposes.

Statistical methods. Standard frequency tables and descriptive statistics were used to characterize the sample data set. The ORR was defined as the best response according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) (38). ORR was estimated using Pearson chi-square test and, in the case where the assumptions of this test were not met, Fisher's exact test. Progression-free survival (PFS) and OS were estimated using Kaplan–Meier method and all point estimates were accompanied by 95% confidence intervals. PFS was determined from the date of nivolumab treatment initiation until the date of first documented progression (as per RECIST 1.1) or death. OS was determined from the date of nivolumab treatment initiation until the date of death due to any cause. Patients whose disease had not progressed or who had died were censored at the date of last follow-up. Statistical significance of the differences in Kaplan–Meier estimates was assessed using the log-rank test. Finally, multivariate Cox proportional hazards model was used to evaluate the effect of all potential prognostic factors on the survival measures. For decision on statistical significance, α=0.05 was used.

Results

Patient characteristics. In total, 224 patients, 133 male and 91 females, with a median age of 67 years, were included in the present retrospective analysis. Among 22 patients treated with corticosteroids, 11 were treated for longer than 2 months followed-up in this study, while the duration of corticosteroid therapy in the remaining 11 patients was shorter (median of 4 days). The median duration of antibiotic therapy among patients treated with antibiotics was 7 days. The principal reason for antibiotic treatment was respiratory infection in 21 patients (77.8%). Other indications for antibiotic use included phlegmon (two patients), prophylaxis (two patients), urinary tract infection (one patient) and cholangitis (one patient). The median length of probiotic treatment among patients using probiotics was 7.5 days. Among patients using PPIs, most (59; 92.2%) were treated for more than 2 months in this retrospective analysis. Among the patients using NSAIDs, the majority (40; 87.0%) used NSAIDs for more than 2 months and all patients used statins or metformin more than 2 months. The baseline patient characteristics, and characteristics of the use of studied concomitant medications, are summarized in Tables I and II.
ORR. Only 181 patients with a known response were included in this analysis. Only the association between ORR and corticosteroids was significant (p=0.019): more progressive disease was found in corticoid users. Among the patients treated with corticosteroids (n=16), partial response was observed in four (25%), stable disease in five (31%), and progressive disease in seven patients (44%). Among patients not treated with corticosteroids (n=165), partial response was seen in 27 (16.4%), stable disease in 106 (64.2%) and progressive disease in 32 (19.4%). There was no relation between ORR and administration of antibiotics (p=0.359), probiotics (p=0.515), PPIs (p=0.655), NSAIDs (p=0.504), metformin (p>0.999) or statins (p=0.875).

Univariate analysis of PFS and OS. There were no significant differences in OS or PFS between the patients taking or not taking the studied concomitant medications (Table III).

Multivariate Cox proportional hazards model. The Cox model only included patients with known value of C-reactive protein (CRP) (210 patients). Age, Eastern Cooperative Oncology Group performance score, histology and CRP concentration were found to be significant predictors of OS. An increase in patient age by 1 year, reduced the risk of death by 1.038-fold. Patients with ECOG PS 1 had a 1.980-fold higher risk of death than those with ECOG PS 0. Patients with squamous cell carcinoma had 1.733-fold lower risk of death than those with adenocarcinoma. Each CRP increase of 1 mg/dl increased the risk of death by 1.016-fold.

In the Cox model for PFS, age, histology, CRP concentration and treatment with NSAID were significant predictors. An increase in patient age by 1 year, reduced the risk of death by 1.028-fold. Patients with squamous cell carcinoma had a 1.689-fold lower risk of progression than those with adenocarcinoma. CRP increase of 1 mg/dl increased the risk of progression by 1.005-fold. Patients not taking NSAIDs had a 1.596-fold higher risk of progression than those taking an NSAID (Table IV).

Discussion

The data from the present retrospective analysis indicate an effect of corticosteroids on ORR and possible impact of NSAID on PFS in patients with NSCLC treated with nivolumab. Some of these results are in conflict with prior reports. We believe that there are more factors that can explain these differences as discussed below.

A number of studies has indicated a possible lower efficacy of immunotherapy when antibiotics are co-administered with nivolumab (17, 20, 21, 39-46). On the other hand, the present results are in accordance with Kaderbhai *et al*., who showed no significant effect of antibiotics on PFS or OS (7). However, that study included 53.3% of patients treated with antibiotics

Table I. *Baseline patient characteristics*.

Parameter	n (%)
Gender	
Male	133 (59.4)
Female	91 (40.6)
Smoking status	
Smoker	109 (48.7)
Former smoker	72 (32.1)
Non-smoker	43 (19.2)
ECOG PS	
0	56 (25)
1	164 (73.2)
2	4 (21.8)
Line of therapy	
First	9 (4)
Second	108 (48.2)
Third	55 (24.6)
Fourth	36 (16.1)
Fifth	14 (6.3)
Sixth	2 (0.9)
Histology	
Adenocarcinoma	137 (61.2)
SCC	86 (38.4)
Other	1 (0.4)
Stage	
III	24 (10.7)
IV	200 (89.3)
CRP	
<10 mg/l	76 (36.2)
≥10 mg/l	134 (63.8)

CRP: C-Reactive protein, ECOG PS: Eastern Cooperative Oncology Group performance status; SCC: squamous cell carcinoma.

Table II. *Characteristics of concomitant medication*.

Parameter	n (%)
Corticosteroid use	
No	202 (90.2)
Yes	22 (9.8)
Corticosteroid medication	
Prednisone	9 (40.9)
Dexamethasone	9 (40.9)
Methylprednisolone	4 (18.2)
Corticosteroid dose (prednisone equivalent)	
>10 mg	14 (63.7)
≤10 mg	5 (22.7)
Unknown	3 (13.6)
Antibiotic	
No	197 (87.9)
Yes	27 (12.1)
Macrolide	4 (14.8)
Amoxicillin/clavulanate	8 (29.7)
Amoxicillin/clavulanate/macrolide	3 (11.1)
Tetracycline	2 (7.4)
Cephalosporin (3rd generation)	4 (14.8)
Ciprofloxacin	6 (22.2)
Route of antibiotic administration	
Intravenous	3 (11.1)
Oral	21 (77.8)
Combined	3 (11.1)
Probiotic (Lactobacillus)	
No	218 (97.3)
Yes	6 (2.7)
PPI	
No	160 (71.4)
Yes	64 (28.6)
Omeprazole	41 (64.1)
Pantoprazole	21 (32.8)
Lansoprazole	2 (3.1)
NSAID	
No	178 (79.5)
Yes	45 (20.5)
Acetylsalicylic acid	11 (23.9)
Nimesulide	10 (21.7)
Ibuprofen	6 (13)
Aceclofenac	6 (13)
Diclofenac	3 (6.5)
Ibuprofen	3 (6.5)
Indomethacin	3 (6.5)
Meloxicam	1 (2.2)
Naproxen	1 (2.2)
Nimesulide + diclofenac	1 (2.2)
Tiaporofenic acid	1 (2.2)
Statin	
No	193 (86.2)
Yes	31 (13.8)
Atorvastatin	24 (77.4)
Rosuvastatin	6 (19.4)
Simvastatin	1 (3.2)
Metformin	
No	206 (92)
Yes	18 (8)

PPI: Proton pump inhibitors; NSAID: non-steroidal anti-inflammatory drug.

more than 1 month prior to starting treatment with nivolumab, in contrast to the present study and many other similar studies. This may have adversely affected the results as it is known that the gut microbiome normalizes after some time subsequently to antibiotic administration (17, 42). A number of factors may explain contradictory results regarding the interaction between antibiotic treatment and cancer immunotherapy. Firstly, the individual studies analyzed patients from different regions and different results may reflect regional differences in the gut microbiome (42). There were also differences with regard to antibiotic use. For example, in the present study, aminopenicillins and macrolides were the most commonly used antibiotics, while in the study reported by Hakozaki *et al*., trimethoprim/sulfamethoxazole was used more frequently, and quinolones were more extensively used in some other studies, specifically in the United States (17, 21, 39, 44). Individual antibiotics are characterized by a spectrum of antimicrobial activity, and only some antibiotics negatively affect the microbiome in terms of immunotherapy efficacy (21). The route of antibiotic administration may also play a role, with oral administration (predominant in the present

Table III. *Univariate analysis of progression-free (PFS) and overall (OS) survival.*

	Median PFS (95% CI), months			Median OS (95% CI), months		
Parameter	Taking drug	Not taking drug	*p*-Value	Taking drug	Not taking drug	*p*-Value
Corticosteroid	3.6 (1.7-NA)	6.0 (4.1-7.0)	0.398	6.2 (3.6-NA)	13.1 (11.3-16.9)	0.485
Antibiotic	4.4 (2.7-10.6)	6.0 (4.0-7.2)	0.152	12.8 (6.1-NA)	13.1 (11.0-20.0)	0.191
Probiotic	6.3 (4.4-NA)	5.9 (3.9-7.0)	0.821	7.5 (6.1-NA)	13.0 (11.0-16.9)	0.837
PPI	3.7 (2.8- 8.8)	6.1 (4.3-7.2)	0.117	9.9 (7.5-33.9)	14.6 (11.7-20.0)	0.101
NSAID	6.9 (4.8-24.9)	5.3 (3.7-6.8)	0.120	16.8 (8.7-NA)	12.8 (9.9-16.8)	0.297
Statin	7.2 (3.2-10.6)	5.4 (3.8-6.9)	0.320	16.8 (9.6-NA)	12.9 (10.6-16.9)	0.426
Metformin	3.3 (2.9-NA)	6.0 (4.1-7.0)	0.562	10.6 (3.3-NA)	13.1 (11.3-17.7)	0.440

PPI: Proton pump inhibitors; NA: not achieved; NSAID: non-steroidal anti-inflammatory drug.

Table IV. *Multivariate Cox proportional hazards model for progression-free (PFS) and overall (OS) survival.*

		OS		PFS	
Category	Category	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Gender	Male	Reference		Reference	
	Female	1.278 (0.779-2.095)	0.331	1.045 (0.714-1.529)	0.822
Age	1-Year increase	0.963 (0.940-0.987)	0.003	0.972 (0.953-0.991)	0.004
Smoking status	Non-smoker	Reference		Reference	
	Former-smoker	1.431 (0.715-2.862)	0.312	1.455 (0.867-2.442)	0.156
	Smoker	0.784 (0.400-1.536)	0.478	0.762 (0.457-1.270)	0.297
ECOG PS	0	Reference		Reference	
	1	1.980 (1.074-3.651)	0.029	1.507 (0.959-2.368)	0.076
	2	0.888 (0.180-4.383)	0.884	1.263 (0.362-4.404)	0.714
Line of therapy	First	Reference		Reference	
	Second	0.884 (0.340-2.297)	0.801	0.805 (0.343-1.892)	0.619
	Third	0.753 (0.286-1.985)	0.567	1.064 (0.445-2.546)	0.888
	Fourth	0.794 (0.292-2.161)	0.651	1.179 (0.476-2.923)	0.722
	Fifth or sixth	0.563 (0.166-1.913)	0.358	0.691 (0.243-1.961)	0.487
Histology*	AC	Reference		Reference	
	SCC	0.577 (0.334-0.995)	0.048	0.592 (0.383-0.914)	0.018
Stage	III	Reference		Reference	
	IV	1.546 (0.743-3.218)	0.244	1.211 (0.689-2.129)	0.505
CRP concentration	1 mg/dl Increase	1.016 (1.010-1.023)	<0.001	1.005 (1.000-1.010)	0.048
Corticosteroid	Yes	Reference		Reference	
	No	0.737 (0.344-1.580)	0.433	0.933 (0.502-1.733)	0.826
Antibiotic	Yes	Reference		Reference	
	No	1.513 (0.717-3.193)	0.277	1.182 (0.642-2.178)	0.591
Probiotic	Yes	Reference		Reference	
	No	1.086 (0.279-4.232)	0.905	0.765 (0.272-2.152)	0.611
PPI	Yes	Reference		Reference	
	No	0.822 (0.487-1.388)	0.463	0.737 (0.485-1.121)	0.154
NSAID	Yes	Reference		Reference	
	No	1.744 (0.946-3.216)	0.075	1.596 (1.018-2.503)	0.042
Statin	Yes	Reference		Reference	
	No	1.324 (0.679-2.584)	0.41	1.251 (0.748-2.093)	0.393
Metformin	Yes	Reference		Reference	
	No	0.521 (0.250-1.089)	0.083	0.714 (0.383-1.329)	0.287

ECOG PS: Eastern Cooperative Oncology Group performance status; AC: adenocarcinoma; SCC: squamous cell carcinoma; CRP: C-reactive protein; PPI: proton pump inhibitors; NSAID: non-steroidal anti-inflammatory drug. *One patient with adeno-squamous cell carcinoma was included in the group of patients with adenocarcinoma.

cases) being potentially associated with improved efficacy of immunotherapy (47). Moreover, a bias cannot be excluded as patients using antibiotics usually have higher concentrations of CRP, which is a negative predictive and prognostic factor. Last but not least, a possible effect of other concomitant drugs that may affect the microbiome (*e.g.* corticosteroids, antacids, or NSAIDs) should not be forgotten (45). All these factors may not have been adequately taken into account in many reports.

The present study demonstrated the effect of corticotherapy on ORR treatment with nivolumab, while other studies showed also it to have an impact on PFS and OS (6, 15). Arbour *et al.* showed a decrease in both PFS and OS in patients treated with corticosteroids at a dose equivalent to ≥10 mg prednisone in comparison with other patients, including patients taking lower doses of corticosteroids (15). Fuca *et al.* also considered only patients with higher corticosteroid doses in their study showing worse outcome in patients treated with corticosteroid together with immunotherapy (8). In the present retrospective analysis, on the other hand, patients taking a low dose of corticosteroid were also considered, which may have affected the results. It is possible that lower doses of corticosteroids administered at the time of initiation of nivolumab therapy have no impact on the outcome. A meta-analysis on this topic also points to other factors that may have a role here (16). In particular, there may be a varying proportion of indications for corticotherapy in individual studies, such as intracranial metastases. The indication for corticotherapy was not analyzed in the present retrospective study, which may be one of its limitations. Finally, the negative results with regard to PFS and OS might easily be explained by the limited number of patients taking corticosteroids.

The results regarding the use of PPIs are consistent with the study by Routy *et al.* that indicated no effect of PPI on the outcome of immunotherapy (20). Similar results were reported by Hakozaki *et al.* in a cohort with a much higher use of PPIs (17), as well as by Zhao *et al.* (44). In the present study, no significant effect of probiotics was evident, but only few patients were taking probiotics. Probiotics are over-the-counter medications and their use might have not been reported by all patients, and, therefore, no definitive conclusions can be drawn.

As already mentioned, statins and metformin may potentiate the effect of anticancer therapy, including immunotherapy (26, 27). In univariate analysis, Omori *et al.* demonstrated significant improvement of the time to treatment failure in patients treated with nivolumab and statins (6), but only 10 patients in the cohort were treated with statins and multivariate analysis was not performed. The present study, which included a higher number of patients taking statins or metformin, did not indicate any effect of these drugs on the efficacy of nivolumab. Interactions between drugs administered simultaneously might also explain differences in the results.

NSAIDs have, among other mechanisms of action, an impact on inflammation by inhibiting COX2. COX2 is associated with PD-L1 expression in NSCLC (37). In a previous study, we demonstrated an association of parameters of inflammatory response with inferior outcome of nivolumab treatment in patients with NSCLC (5). A correlation between the concentrations of CRP, a biomarker of inflammatory response, and PFS and OS was evident in multivariate analysis of patients with NSCLC treated with nivolumab. Taking NSAIDs in the present study was associated with improved PFS in the multivariate analysis. Therefore, a positive immunomodulatory effect of NSAID on immunotherapy cannot be excluded. No such effect was evident in the univariate analyses; the effect might have been masked by other factors, including concomitant medications. Further larger prospective studies, as well as preclinical research on this topic, is therefore necessary.

The present study has several limitations. Firstly, this was a retrospective study of patients. Secondly, PFS was not confirmed by an independent review. Thirdly, the majority of patients had no PD-L1 status reported as this test was not necessary for the administration of nivolumab but PD-L1 status is associated with nivolumab efficacy in patients with non-squamous histology (2). Lastly, but not least, many analyses lacked sufficient statistical power as the number of patients taking some of concomitant medications was limited. Thus, the present report should be regarded as exploratory and the results should be confirmed in a larger prospective study.

In conclusion, the results of the present retrospective exploratory analysis underscore the importance of detailed knowledge of concomitant medications, including the route of administration and dose, in evaluating the effect on the outcome of nivolumab therapy. In the present study, a significantly higher progression rate was evident in patients treated with corticosteroids, and a positive effect of NSAID use at the time initiation of nivolumab treatment was observed.

Conflicts of Interest

In connection with this article, the Authors declare consulting services for BMS in the past.

Authors' Contributions

MS and JB conceived the presented idea. MS, MZ, PZ, JK, OF, JS, LJ, MC, MH, MJ, JK, DK, OB and BM conceived and planned the experiments and collected the data. KH and MB analyzed the data. MS wrote the article with support from JB and BM. BM helped supervise the project.

Acknowledgements

This work was supported by AZV grant No. 17-30748A.

References

1 Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Arén Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B and Spigel DR: Nivolumab *versus* docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med *373(2)*: 123-135, 2015. PMID: 26028407. DOI: 10.1056/NEJMoa1504627

2 Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhäufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crinò L, Blumenschein GR Jr, Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F and Brahmer JR: Nivolumab *versus* docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med *373(17)*: 1627-1639, 2015. PMID: 26412456. DOI: 10.1056/NEJMoa1507643

3 Brody R, Zhang Y, Ballas M, Siddiqui MK, Gupta P, Barker C, Midha A and Walker J: PD-L1 expression in advanced NSCLC: Insights into risk stratification and treatment selection from a systematic literature review. Lung Cancer *112:* 200-215, 2017. PMID: 29191596. DOI: 10.1016/j.lungcan.2017.08.005

4 Penault-Llorca F and Radosevic-Robin N: Tumor mutational burden in non-small cell lung cancer–the pathologist's point of view. Transl Lung Cancer Res *7(6)*: 716-721, 2018. PMID: 30505716. DOI: 10.21037/tlcr.2018.09.26

5 Svaton M, Zemanova M, Skrickova J, Jakubikova L, Kolek V, Kultan J, Koubkova L, Bejckova A, Salajka F, Hrnciarik M, Melichar B, Vrana D, Konecny M, Chloupkova R and Pesek M: Chronic inflammation as a potential predictive factor of nivolumab therapy in non-small cell lung cancer. Anticancer Res *38(12)*: 6771-6782, 2018. PMID: 30504389. DOI: 10.21873/anticanres.13048

6 Omori M, Okuma Y, Hakozaki T and Hosomi Y: Statins improve survival in patients previously treated with nivolumab for advanced non-small cell lung cancer: An observational study. Mol Clin Oncol *10(1)*: 137-143, 2019. PMID: 30655989. DOI: 10.3892/mco.2018.1765

7 Kaderbhai C, Richard C, Fumet JD, Aarnink A, Foucher P, Coudert B, Favier L, Lagrange A, Limagne E, Boidot R and Ghiringhelli F: Antibiotic use does not appear to influence response to nivolumab. Anticancer Res *37(6)*: 3195-3200, 2017. PMID: 28551664. DOI: 10.21873/anticanres.11680

8 Fucà G, Galli G, Poggi M, Lo Russo G, Proto C, Imbimbo M, Ferrara R, Zilembo N, Ganzinelli M, Sica A, Torri V, Colombo MP, Vernieri C, Balsari A, de Braud F, Garassino MC and Signorelli D: Modulation of peripheral blood immune cells by early use of steroids and its association with clinical outcomes in patients with metastatic non-small cell lung cancer treated with immune checkpoint inhibitors. ESMO Open *4(1)*: e000457, 2019. PMID: 30964126. DOI: 10.1136/esmoopen-2018-000457

9 Ryken TC, McDermott M, Robinson PD, Ammirati M, Andrews DW, Asher AL, Burri SH, Cobbs CS, Gaspar LE, Kondziolka D, Linskey ME, Loeffler JS, Mehta MP, Mikkelsen T, Olson JJ, Paleologos NA, Patchell RA and Kalkanis SN: The role of steroids in the management of brain metastases: A systematic review and evidence-based clinical practice guideline. J Neurooncol *96(1)*: 103-114, 2010. PMID: 19957014. DOI: 10.1007/s11060-009-0057-4

10 Paulsen O, Klepstad P, Rosland JH, Aass N, Albert E, Fayers P and Kaasa S: Efficacy of methylprednisolone on pain, fatigue, and appetite loss in patients with advanced cancer using opioids: A randomized, placebo-controlled, double-blind trial. J Clin Oncol *32(29)*: 3221-3228, 2014. PMID: 25002731. DOI: 10.1200/JCO.2013.54.3926

11 Libert C and Dejager L: How steroids steer T-cells. Cell Rep *7(4)*: 938-939, 2014. PMID: 24856295. DOI: 10.1016/j.celrep.2014.04.041

12 Tetel MJ, de Vries GJ, Melcangi RC, Panzica G and O'Mahony SM: Steroids, stress and the gut microbiome–brain axis. J Neuroendocrinol *30(2)*, 2018. PMID: 29024170. DOI: 10.1111/jne.12548

13 Santini FC, Rizvi H, Plodkowski AJ, Ni A, Lacouture ME, Gambarin-Gelwan M, Wilkins O, Panora E, Halpenny DF, Long NM, Kris MG, Rudin CM, Chaft JE and Hellmann MD: Safety and efficacy of re-treating with immunotherapy after immune-related adverse events in patients with NSCLC: Cancer Immunol Res *6(9)*: 1093-1099, 2018. PMID: 29991499. DOI: 10.1158/2326-6066.CIR-17-0755

14 Bagley SJ, Kothari S, Aggarwal C, Bauml JM, Alley EW, Evans TL, Kosteva JA, Ciunci CA, Gabriel PE, Thompson JC, Stonehouse-Lee S, Sherry VE, Gilbert E, Eaby-Sandy B, Mutale F, DiLullo G, Cohen RB, Vachani A and Langer CJ: Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. Lung Cancer *106*: 1-7, 2017. PMID: 28285682. DOI: 10.1016/j.lungcan.2017.01.013

15 Arbour KC, Mezquita L, Long N, Rizvi H, Auclin E, Ni A, Martínez-Bernal G, Ferrara R, Lai WV, Hendriks LEL, Sabari JK, Caramella C, Plodkowski AJ, Halpenny D, Chaft JE, Planchard D, Riely GJ, Besse B and Hellmann MD: Impact of baseline steroids on efficacy of programmed cell death-1 and programmed death-ligand 1 blockade in patients with non-small-cell lung cancer. J Clin Oncol *36(28)*: 2872-2878, 2018. PMID: 30125216. DOI: 10.1200/JCO.2018.79.0006

16 Garant A, Guilbault C, Ekmekjian T, Greenwald Z, Murgoi P and Vuong T: Concomitant use of corticosteroids and immune checkpoint inhibitors in patients with hematologic or solid neoplasms: A systematic review. Crit Rev Oncol Hematol *120*: 86-92, 2017. PMID: 29198341. DOI: 10.1016/j.critrevonc.2017.10.009

17 Hakozaki T, Okuma Y, Omori M and Hosomi Y: Impact of prior antibiotic use on the efficacy of nivolumab for non-small cell lung cancer. Oncol Lett *17(3)*: 2946-2952, 2019. PMID: 30854072. DOI: 10.3892/ol.2019.9899

18 Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillère R, Hannani D, Enot DP, Pfirschke C, Engblom C, Pittet MJ, Schlitzer A, Ginhoux F, Apetoh L, Chachaty E, Woerther PL, Eberl G, Bérard M, Ecobichon C, Clermont D, Bizet C, Gaboriau-Routhiau V, Cerf-Bensussan N, Opolon P, Yessaad N, Vivier E, Ryffel B, Elson CO, Doré J, Kroemer G, Lepage P, Boneca IG, Ghiringhelli F and Zitvogel L: The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science *342(6161)*: 971-976, 2013. PMID: 24264990. DOI: 10.1126/science.1240537

19 Vétizou M, Pitt JM, Daillère R, Lepage P, Waldschmitt N, Flament C, Rusakiewicz S, Routy B, Roberti MP, Duong CP, Poirier-Colame V, Roux A, Becharef S, Formenti S, Golden E, Cording S, Eberl G, Schlitzer A, Ginhoux F, Mani S, Yamazaki T, Jacquelot N, Enot DP, Bérard M, Nigou J, Opolon P,

Eggermont A, Woerther PL, Chachaty E, Chaput N, Robert C, Mateus C, Kroemer G, Raoult D, Boneca IG, Carbonnel F, Chamaillard M and Zitvogel L: Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science *350(6264)*: 1079-1084, 2015. PMID: 26541610. DOI: 10.1126/science.aad1329

20 Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, Fluckiger A, Messaoudene M, Rauber C, Roberti MP, Fidelle M, Flament C, Poirier-Colame V, Opolon P, Klein C, Iribarren K, Mondragón L, Jacquelot N, Qu B, Ferrere G, Clémenson C, Mezquita L, Masip JR, Naltet C, Brosseau S, Kaderbhai C, Richard C, Rizvi H, Levenez F, Galleron N, Quinquis B, Pons N, Ryffel B, Minard-Colin V, Gonin P, Soria JC, Deutsch E, Loriot Y, Ghiringhelli F, Zalcman G, Goldwasser F, Escudier B, Hellmann MD, Eggermont A, Raoult D, Albiges L, Kroemer G and Zitvogel L: Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science *359(6371)*: 91-97, 2018. PMID: 29097494. DOI: 10.1126/science.aan3706

21 Ahmed J, Kumar A, Parikh K, Anwar A, Knoll BM, Puccio C, Chun H, Fanucchi M and Lim SH: Use of broad-spectrum antibiotics impacts outcome in patients treated with immune checkpoint inhibitors. Oncoimmunology *7(11)*: e1507670, 2018. PMID: 30377571. DOI: 10.1080/2162402X.2018.1507670

22 Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG and Choi Y: Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature *460(7251)*: 103-107, 2009. PMID: 19494812. DOI: 10.1038/nature08097

23 Rolf J, Zarrouk M, Finlay DK, Foretz M, Viollet B and Cantrell DA: AMPKα1: A glucose sensor that controls CD8 T-cell memory. Eur J Immunol *43(4)*: 889-896, 2013. PMID: 23504580. DOI: 10.1002/eji.201343483

24 Araki K and Ahmed R: AMPK: a metabolic switch for $CD8^+$ T-cell memory. Eur J Immunol *43(4)*: 878-881, 2013. PMID: 23504580. DOI: 10.1002/eji.201343483

25 Scharping NE, Menk AV, Whetstone RD, Zeng X and Delgoffe GM: Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. Cancer Immunol Res *5(1)*: 9-16, 2017. PMID: 27941003. DOI: 10.1158/2326-6066.CIR-16-0103.

26 Gruenbacher G and Thurnher M: Mevalonate metabolism in cancer. Cancer Lett *356(2 Pt A)*: 192-196, 2015. PMID: 24467965. DOI: 10.1016/j.canlet.2014.01.013

27 Fiala O, Pesek M, Finek J, Minarik M, Benesova L, Bortlicek Z and Topolcan O: Statins augment efficacy of EGFR-TKIs in patients with advanced-stage non-small cell lung cancer harbouring *KRAS* mutation. Tumour Biol *36(8)*: 5801-5805, 2015. PMID: 25702091. DOI: 10.1007/s13277-015-3249-x

28 Chan AT, Ogino S and Fuchs CS: Aspirin and the risk of colorectal cancer in relation to the expression of COX2. N Engl J Med *356(21)*: 2131-2142, 2007. PMID: 17522398. DOI: 10.1056/NEJMoa067208

29 Bucher C, Jordan P, Nickeleit V, Torhorst J and Mihatsch MJ: Relative risk of malignant tumors in analgesic abusers. Effects of long-term intake of aspirin. Clin Nephrol *51(2)*: 67-72, 1999. PMID: 10069640.

30 Thun MJ, Henley SJ and Gansler T: Inflammation and cancer: an epidemiological perspective. Novartis Found Symp *56*: 6–21, 2004. PMID: 15027481.

31 Hla T and Neilson K: Human cyclooxygenase-2 cDNA: Proc Natl Acad Sci USA *89(16)*: 7384-7388, 1992. PMID: 1380156. DOI: 10.1073/pnas.89.16.7384

32 Sobolewski C, Cerella C, Dicato M, Ghibelli L and Diederich M: The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol *2010*: 215158, 2010. PMID: 20339581. DOI: 10.1155/2010/215158

33 Sabbatino F, Wang Y, Scognamiglio G, Favoino E, Feldman SA, Villani V,Flaherty KT, Nota S, Giannarelli D and Simeone E: Antitumor activity of BRAF inhibitor and IFNalpha combination in *BRAF*-mutant melanoma. J Natl Cancer Inst *108*: 435, 2016. PMID: 26851802. DOI: 10.1093/jnci/djv435

34 Botti G, Fratangelo F, Cerrone M, Liguori G, Cantile M, Anniciello AM, Scala S, D'Alterio C3, Trimarco C, Ianaro A, Cirino G, Caracò C, Colombino M, Palmieri G, Pepe S, Ascierto PA, Sabbatino F and Scognamiglio G: COX2 expression positively correlates with PD-L1 expression in human melanoma cells. J Transl Med *15(1)*: 46, 2017. PMID: 28231855. DOI: 10.1186/s12967-017-1150-7

35 Liang M, Yang H and Fu J: Nimesulide inhibits IFN-gamma-induced programmed death-1-ligand 1 surface expression in breast cancer cells by COX2 and PGE_2 independent mechanisms. Cancer Lett *276(1)*: 47-52, 2009. PMID: 19046800. DOI: 10.1016/j.canlet.2008.10.028

36 Hamada T, Cao Y, Qian ZR, Masugi Y, Nowak JA, Yang J, Song M, Mima K, Kosumi K, Liu L, Shi Y, da Silva A, Gu M, Li W, Keum N, Zhang X, Wu K, Meyerhardt JA, Giovannucci EL, Giannakis M, Rodig SJ, Freeman GJ, Nevo D, Wang M, Chan AT, Fuchs CS, Nishihara R and Ogino S: Aspirin use and colorectal cancer survival according to tumor CD274 (programmed cell death 1 ligand 1) expression status. J Clin Oncol *35(16)*: 1836-1844, 2017. PMID: 28406723. DOI: 10.1200/JCO.2016.70.7547

37 Shimizu K, Okita R, Saisho S, Maeda AI, Nojima Y and Nakata M: Impact of COX2 inhibitor for regulation of PD-L1 expression in non-small cell lung cancer. Anticancer Res *38(8)*: 4637-4644, 2018. PMID: 30061230. DOI: 10.21873/anticanres.12768

38 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst *92*: 205-216, 2000. PMID: 10655437. DOI: 10.1093/jnci/92.3.205

39 Thompson J, Szabo A, Arce-Lara C and Menon S: P1. 07-008 Microbiome & immunotherapy: Antibiotic use is associated with inferior survival for lung cancer patients receiving PD-1 Inhibitors. J Thorac Oncol *12(11)*: S1998, 2017. DOI: 10.1016/j.jtho.2017.09.926

40 Do TP, Hegde AM, Cherry CR, Chipman Robert Geoffrey Stroud CRG, Sharma N, Cherukuri SD, Bowling M and Walker PR: Antibiotic use and overall survival in lung cancer patients receiving nivolumab. J Clin Oncol *36*:15_suppl, e 15109-e 15109, 2018. DOI: 10.1200/JCO.2018.36.15_suppl.e15109

41 Tinsley N, Zhou C, Villa S, Tan G, Lorigan P, Blackhall FH, Elliott T, Krebs M, Carter L, Thistlethwaite M, Hughes A and Cook N: Cumulative antibiotic use and efficacy of immune checkpoint inhibitors in patients with advanced cancer. J Clin Oncol *36(15_suppl)*: 3010-3010, 2018. DOI: 10.1200/JCO.2018.36.15_suppl.3010

42 Derosa L, Hellmann MD, Spaziano M, Halpenny D, Fidelle M, Rizvi H, Long N, Plodkowski AJ, Arbour KC, Chaft JE, Rouche

JA, Zitvogel L, Zalcman G, Albiges L, Escudier B and Routy B: Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann Oncol *29(6)*: 1437-1444, 2018. PMID: 2961771. DOI: 10.1093/annonc/mdy103

43 Ouaknine Krief J, Helly de Tauriers P, Dumenil C, Neveux N, Dumoulin J, Giraud V, Labrune S, Tisserand J, Julie C, Emile JF, Chinet T and Giroux Leprieur E: Role of antibiotic use, plasma citrulline and blood microbiome in advanced non-small cell lung cancer patients treated with nivolumab. J Immunother Cancer *7(1)*: 176, 2016. PMID: 31292005. DOI: 10.1186/s40425-019-0658-1

44 Zhao S, Gao G, Li W, Li X, Zhao C, Jiang T, Jia Y, He Y, Li A, Su C, Ren S, Chen X and Zhou C: Antibiotics are associated with attenuated efficacy of anti-PD-1/PD-L1 therapies in Chinese patients with advanced non-small cell lung cancer. Lung Cancer *130*: 10-17, 2019. PMID: 30885328. DOI: 10.1016/j.lungcan.2019.01.017

45 A Schett, S I Rothschild, L A Mauti, S Schmid, C Appenzeller, A Curioni-Fontecedro, M Frueh and M Joerger: 157P Prognostic impact of the use of antibiotics in patients with advanced non-small cell lung cancer (NSCLC) receiving PD-(L)1 targeting monoclonal antibodies. Ann Oncol *30 (suppl_2):* ii38-ii68, 2019. DOI: 10.1093/annonc/mdz063

46 Elkrief A, El Raichani L, Richard C, Messaoudene M, Belkaid W, Malo J, Belanger K, Miller W, Jamal R, Letarte N, Wong P and Routy B: Antibiotics are associated with decreased progression-free survival of advanced melanoma patients treated with immune checkpoint inhibitors. Oncoimmunology *8(4)*: e1568812, 2019. PMID: 30906663. DOI: 10.1080/2162402X.2019.1568812

47 Mielgo Rubio X, Aguado C, Sereno M, Chara LE, Cabezón L, Velastegui A, López A, Lopez Castro R, Sotelo M, Rubio J, Nunez JA, Pérez Fernández E, Olier C, De Castro Carpeno J, Antonanzas M, Falagan S, Gomez-Barreda I, Custodio S and Cruz P: P1. 04-16: Early antibiotic use affects the efficacy of first-line immunotherapy in lung cancer patients but route of administration seems to be decisive. J Thorac Oncol*14(10)*: S445, 2019. DOI: 10.1016/j.jtho.2019.08.919

Received February 6, 2020
Revised February 24, 2020
Accepted February 29, 2020